FINAL REPORT

South Carolina State Wildlife Grant SC-T-F12AF01381 (T-59-R-1)
South Carolina Department of Natural Resources
October 1, 2012 – September 30, 2015

NOTE: This grant received a one-year, no-cost extension for its current end date.

Project Title:
AMERICAN EEL POPULATIONS IN SOUTH CAROLINA AND THEIR INFECTION BY INVASIVE PARASITES

Job 1:
Quantify infections by the invasive swim bladder parasite *Anguillicoloides crassus* (= *Anguillicola crassus*) in South Carolina American eel populations (SC 2005 CWCS pp. 2-2, 2-23, 4-25).

Objective 1.1:
Determine the percentage of eels infected by *A. crassus* in South Carolina populations of American eel.

Accomplishments:
We collected a total of 1,150 American eels (*Anguilla rostrata*) from 183 sites covering 11 river drainages in South Carolina (**Table 1**; **Figure 1**). Six hundred and seventy seven of the eels were yellow or silver eels collected by electrofishing. The remaining 473 eels were juveniles (glass eels, n = 274; elvers, n = 199) collected from an eel ladder on the dam of Goose Creek Reservoir, which flows via Goose Creek into the Cooper River, near North Charleston (the eel ladder is maintained by the Diadromous Section of SCDNR).

Yellow and silver eel stages were collected during all months of the year, whereas glass eel and elver stages were collected during March through December (**Table 2**). All eels were returned to the laboratory on ice (yellow/silver eels) or alive in water (juveniles). When possible, they were dissected within 24 hours of collection. Otherwise, they were preserved at -20^{o}C and dissected at a later time, after thawing.

Eels were dissected to screen for infections of the swimbladder parasite *A. crassus*, which is an invasive nematode species from Asia that infects anguillid eels. The *A. crassus* parasite infects eels by being ingested as an L_3 larva living inside an intermediate or paratenic host. The parasite migrates to the eel's swimbladder wall and transforms into an L_4 larva. It then transforms into an adult worm that lives in the swimbladder lumen.

Yellow & Silver Eels: Yellow and silver eels ranged from 109 to 798 mm total length (**Figure 2**). We macroscopically screened 670 yellow/silver eels for adult stages of the parasite, and microscopically (x 400 magnification) screened 615 of them for L_3/L_4 larval stages. The maximum number of *A. crassus* parasites found per eel was 45 for the L_3/L_4 larval parasites, 63 for adult parasites, and 89 for larval and adult parasites combined. Information on the prevalence, mean abundance and mean intensity of infection is summarized in **Table 3**.

Juvenile eels (glass eels and elvers): Juvenile eels ranged from 34 to 156 mm total length (**Figure 3**). We screened all 473 of the eels under a microscope for both the L_3/L_4 and adults stage of the *A. crassus* parasite. In total, 29.4 % of the eels were infected by any parasite stage, with a mean abundance of 0.9 parasites per eel and a mean intensity of 3.0 parasites per infected eel. Infection generally increased with progression of eel development stage (**Table 4**, **Figure 4**). This finding is important because the Cooper River drainage, where our study was performed, is the only area in South Carolina with a licensed glass eel fishery. Our findings indicate that transport of live glass eels and elvers from the Cooper River system could spread the parasite to other areas. These findings were presented at the American Fisheries Society annual meeting in Quebec (2013) and were published in a special edition of the ICES Journal of Marine Sciences (Hein et al., 2015).

Paratenic hosts: During our project, an opportunity arose to investigate paratenic hosts of *A. crassus* parasites in South Carolina through a collaboration with colleagues in Canada. Paratenic hosts are organisms that are not necessary for a parasite to complete its life cycle, and in which the parasite is unable to develop or reproduce. They may nevertheless transmit the parasite to other hosts.

We collected 181 small (TL < 13 cm) fish belonging to 18 different species from two stretches of the Ashley River and one stretch of the Cooper River, South Carolina. All three areas overlapped our yellow/silver eel collections sites, where *A. crassus* was known to occur (see above). Our colleagues screened the fish for *A. crassus* parasites and found that four species were infected, including spot (*Leiostomus xanthurus*), silver perch (*Bairdiella chrysoura*), highfin goby (*Gobionellus oceanicus*) and mummichog (*Fundulus heteroclitus*) (**Table 5**). Our study, which was recently published (Li et al., 2015), provides the first record of paratenic hosts for *A. crassus* in North America.

Significant deviations: In addition to the tasks outlined in our original proposal, we screened juvenile stages of American eel from the Goose Creek Reservoir eel ladder, and 18 putative paratenic host species.

Objective 1.2:
Additionally, determine the severity of infection (e.g. number of parasites per eel; severity of swim bladder damage).

Accomplishments:
Information on the severity of infection (prevalence, abundance and intensity) by *A. crassus* parasites is presented under Objective 1.1 (above) and in **Table 3**, **Table 4** and **Table 5**.

Swim bladder damage: To assess the degree of swimbladder damage caused by *A. crassus* parasites, we used a 'swimbladder degenerative index' (SDI) devised by Lefebvre et al. (2002). The score is based on: (i) opacity of the swimbladder wall (with swimbladder opened), (ii) presence of blood and/or exudate in the lumen of the swimbladder (examined under the dissecting microscope), and (iii) thickness of the swimbladder wall (measured with calipers to the nearest mm). Each component of the index was scored as 0 (least damaged), 1 or 2 (most damaged), and SDI was then calculated as the sum of the three components, ranging from 0-6. Lefebvre et al. (2002) considered SDI ≥ 4 as representative of severe swimbladder damage.

Severe damage is probably indicative of poor reproductive success since swim bladder function is important during the eel's long migration to the Sargasso Sea spawning grounds (~1,000 km for South Carolina eels).

SDI was scored in 576 of the yellow/silver eels. The mean score was 1.3, with the majority of eels (68.4%) having scores of 0 or 1. However, 11.8% of the eels had severe swimbladder damage (SDI $\geq$ 4), suggesting that their ability to migrate and spawn was likely compromised by infection of *A. crassus* parasites.

To assess whether swimbladder damage was affected by eel total length, eels were categorized into small (TL < 310 mm, n = 192), medium (TL = 310-446 mm, n = 192) or large (TL > 446 mm, n = 192), and swim bladder damage was categorized as light (SDI = 0-1, n = 394), moderate (SDI = 2-3, n = 114) or severe (SDI $\geq$ 4, n = 68). Severe swim bladder damage occurred among all eel sizes, but was marginally more common in the largest eels (Chi square test, p = 0.036; **Figure 5**).

To assess whether SDI varied among river systems, we focused our analysis on the Ashley River (n = 105 eels), Cooper River (n = 152), Little Pee Dee (n = 135) and Winyah Bay (n = 74), since they provided sufficient sample sizes for comparison. A marginal difference was detected among systems, with swim bladder damage being least severe in the Little Pee Dee and most severe in the Copper River and Winyah Bay (**Figure 6**).

Significant deviations: None.

Job 2:
Determine the sex and maturity of American eels in different areas of South Carolina (SC 2005 CWCS pp. 2-2, 2-21, 2-23, AP1-7).

Objective 2.1:
Determine the sex ratio among South Carolina populations of American eel.

Accomplishments:
We prepared histological slides of gonad tissue and then microscopically assessed the sex of 231 American eels collected during October 2012 through October 2013. The eels came from the Ashley River (n = 38), Combahee River (n = 55), Cooper River (n = 77), Edisto River, (n = 20) and Winyah Bay (n = 41). Sex was undifferentiated in 49 of the eels (i.e. the gonads were either too small to isolate, or sexual features were indistinguishable when histologically prepared gonad samples were examined under a microscope). Among the remaining eels, which were all examined histologically by two independent readers, there were 53 males and 129 females.

The proportion of males and females varied significantly with eel total length and time of year (ordinal regression, p < 0.001). In general, males were smaller than females and they occurred less frequently than females, especially during the period April-June (**Figure 7**).

Significant deviations: None.

Objective 2.2:
Determine the maturity status among South Carolina populations of American eel.

Accomplishments:
Maturity was categorized in 230 of the eels that were used for determining sex (Objective 2.1). Potential maturity categories included: immature, developing, ripe, or spent (mature eels were those that were developing, ripe or spent). Eels in which sex was undifferentiated were classified as immature (n = 49).

None of the eels was ripe or spent, which is to be expected because the final stages of maturation and spawning occur in the Atlantic Ocean. Among the males, 4 were immature and 49 were mature (developing). Among the females, 50 were immature and 78 were mature (developing).

Additional analyses of the maturity data are presented under Objective 4.1.

Significant deviations: None.

Job 3:
Determine ages of American eels in South Carolina (SC 2005 CWCS pp. 2-2, 2-21, 2-23, AP1-7).

Objective 3.1:
Validate otolith ageing methods in South Carolina populations of American eel.

Accomplishments:
We used marginal increment analysis (Campana 2001) to infer the number of annuli deposited in eel otoliths over the course of a year. Marginal increment analysis is a method that examines the distance from the edge of the most recently deposited (outermost) annulus to the edge of the otolith. This distance increases as the otolith grows, but declines suddenly at the time of year when a new annulus is deposited. Populations showing a single decline over the duration of a year are assumed to deposit just one annulus per year. This information is critical when inferring age from annuli counts.

The marginal increment was measured (x400 magnification, Image Pro software) in 348 sectioned and polished otoliths. Small otoliths lacking their first annulus were excluded. Otoliths were collected each month of the year, although only one was collected during February, so it was pooled with January data **(Table 6**). A general linear model was used to test for differences in marginal increment width between months, controlling for the number of annuli in the otolith (since otolith growth rate decreases with age). The model confirmed that the marginal increment differed significantly between months ($p = 0.008$), and that the eel population as a whole showed just a single decrease in marginal increment width during the period May through August. This implies that just a single annulus is deposited per year in South Carolina eel otoliths (**Figure 8**).

To determine the repeatability of our annuli count methodology, two readers independently assessed 274 otoliths. This resulted in a final dataset of 268 consensus reads and 6 discarded otoliths (unreadable or no consensus). The readers had absolute agreement (i.e. same counts) in 68% of the otoliths, and agreement within 1 annulus in 94% of otoliths. There was no evidence of systematic bias between readers (**Figure 9**, **Figure 10**).

Significant deviations: None.

Objective 3.2:
Determine the age distribution at different times of the year in South Carolina populations of American eel.

Accomplishments 3.2:
We used annuli counts from yellow and silver eels as a proxy for age (n.b. true age is assumed to as [annuli + 2] years). Annuli counts varied from 0 to 10, and their frequency distribution was similar at different times of the year (**Figure 11**).

Significant deviations 3.2: None.

Job 4:
Integrative analysis of eel sex, age, growth, maturity and parasite data (SC 2005 CWCS pp. 2-2, 2-21, 2-23, AP1-7).

Objectives 4.1:
Analyze whether parasite infection status is affected by spatial factors (e.g. drainage), temporal factors (e.g. year-to-year), environmental factors (e.g. salinity), and biological factors (e.g. eel sex, age and maturity)

Accomplishments 4.1:
We used logistic models to test whether the prevalence of adult *A. crassus* parasites in yellow and silver eels was affected by the covariates eel total length and salinity, and by the categorical factors rivers system, year and quarterly season. We focused our analyses on the Ashley, Combahee, Cooper, Edisto, Little Pee Dee and Winyah Bay systems, where most of our eels were collected, and incorporated some additional data from a previous Master's thesis (Hein 2012), giving us a final dataset of 900 eels spanning October 2010 through February 2015.

Parasite prevalence decreased significantly with eel length ($p = 0.033$), and differed significantly between rivers systems ($p = 0.018$), years ($p = 0.001$) and quarterly seasons ($p = 0.021$). Pairwise comparisons indicated that parasite prevalence was (i) significantly higher in the Cooper and Ashley Rivers compared to the Combahee and Edisto Rivers, (ii) significantly lower in 2011 compared with 2010, 2012 and 2013, and (iii) significantly greater during the second quarter of the year compared with the third and fourth quarters.

Salinity had no significant effect on parasite prevalence ($p = 0.193$) and was not included in the final model.

A subsequent model with just male and female data found no significant effect of sex ($p = 0.841$).

Significant deviations 4.1: None.

Objective 4.2:
Model the relationships between age-at-maturity, size-at-maturity, and length-at-age on a spatially resolved level for both male and female eels.

Accomplishments 4.2:
Size at maturity: We used logistic regressions with binary immature/mature response data to assess relationships between maturity and eel total length. We ran an initial model with both male and female data to test the effects of eel total length (covariate), sex (categorical factor), and quarterly season (Jan-Mar, Apr-Jun, etc.; categorical factor). Maturity increased significantly with length ($p < 0.001$) and occurred at a greater length in females than males ($p < 0.001$). Season had no significant effect in the model ($p = 0.22$).

Logistic models were then fit separately to the male and the female maturity data. Season was not included in either model because its effects were not significant ($p > 0.9$ and $p = 0.19$ in the male and female models, respectively). For males, $TL_{50\%}$ (total length at which 50% of eels was mature) was 212 mm, and $TL_{5\%}$ and $TL_{95\%}$ were 113 and 310 mm, respectively. For females, $TL_{50\%}$ occurred at 379 mm, and $TL_{5\%}$ and $TL_{95\%}$ were 272 and 487 mm, respectively (**Figure 12**). Confidence intervals were quite wide for the male data because most of the males we captured were mature (i.e. few immature data for the model to fit).

Age at maturity: We used logistic models to analyze age at maturity using annuli counts as a proxy for age. Season was dropped from the models because its effects were not significant ($p = 0.75$). Age at maturity differed significantly between males and females ($p < 0.001$). Among just the males, 92% of which were mature, annuli had no significant effect on the proportion of mature eels ($p = 0.818$) (**Figure 13a**). With females, the proportion of mature eels increased significantly with annuli count ($p < 0.001$). Female age at 50% maturity ($A_{50\%}$) occurred at 3.4 annuli, and $A_{95\%}$ occurred at 6.4 annuli (**Figure 13b**).

Spatial effects on maturity: We tested for differences in length and age at maturity between the Combahee River, Edisto River, Ashley River, Cooper River and Winyah Bay systems. To do this, we used separate logistic models for male and female data. In each model, length and annuli were entered as coavariates, and season and river system were entered as categorical factors. Models were simplified by backward elimination, retaining significant parameters ($p \leq 0.05$).

For males, there was insufficient data to test for differences between river systems because most of the individuals we caught were mature (i.e. too few immature data for the model to fit).

For females, length ($p < 0.001$) and river system ($p = 0.04$) were retained in the final model. Pairwise comparisons indicated that length at maturity was smaller in the Combahee River ($TL_{50\%}$ = 326 mm) and Winyah Bay (344 mm) compared with the Ashley (436 mm) and Cooper (436 mm) Rivers.

Growth (length at age): Growth was modeled using the following formulation of the von Bertalanffy growth model:

$$L_t = L_\infty[1 - e^{-K(t-t_0)}]$$

where $L_\infty$is maximum eel total length (mm), *K* is a growth rate parameter, *t* is the number of annuli (age proxy) and t_0 is the intercept at *t* = 0. Model parameters were optimized separately for male and female data (pooled across river systems) by minimizing the sum of squared residuals. The optimized parameters were:

Females: $L_\infty = 2.38942^{10}$, $K = 0.00016$, $t_0 = -6.69335$
Males: $L_\infty = 332.85$, $= 0.53142$, $t_0 = -1.31273$

When female L_∞ was constrained to a more realistic value of TL = 1,000 mm, the remaining two freely estimate female parameters were: $K = 0.06689$ and $t_0 = -4.04412$.

Females grew substantially larger than males, but there was no clear difference in growth between river systems (**Figure 14**).

Significant deviations 4.2: None.

Literature Cited:

Campana, S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology*, 2001; 59:197-242.

Hein JL (2013). *Anguillicoloides crassus*, an invasive parasite of the American eel, *Anguilla rostrata*: population dynamics in South Carolina estuaries and health impacts on the host. Master's Thesis, College of Charleston, SC.

Hein JL, Roumillat WA, Post WC, Hazel AP, de Buron I & Arnott SA (2015). Infection of newly recruited American eels (*Anguilla rostrata*) by the invasive swimbladder parasite *Anguillicoloides crassus* in a U.S. Atlantic tidal creek. *ICES Journal of Marine Sciences*. doi: 10.1093/icesjms/fsv097

Lefebvre F, Contournet P, & Crivelli AJ. (2002). The health state of the eel swimbladder as a measure of parasite pressure by *Anguillicola crassus*. *Parasitology*, 124: 457-463.

Li W, Arnott SA, Jones KM, Braicovich P, de Buron I, Wang GT & Marcogliese DJ (2015). First record of paratenic hosts of the swimbladder nematode *Anguillicola crassus* in North America. *Journal of Parasitology*, 101: 529-535. http://dx.doi.org/10.1645/15-774

Estimated Federal Cost: $97,824 federal; $52,679 match

Recommendations: Close the grant.

Table 1
Number of eels collected from different river systems in South Carolina.

	Eel life history stage		
System	*Yellow/Silver*	*Glass/Elver*	**TOTAL**
Ashley River	105	-	105
Combahee River	102	-	102
Congaree River	1	-	1
Cooper River	152	473	625
Edisto River	44	-	44
Great Pee Dee River	19	-	19
Lower Pee Dee River	135	-	135
Sampit River	7	-	7
Santee River	32	-	32
Savannah River	6	-	6
Winyah Bay	74	-	74
TOTAL	**677**	**473**	**1,150**

Table 2
Number of eels caught in different months of the year.

	Eel life history stage		
Month	*Yellow/Silver*	*Glass/Elver*	**TOTAL**
Jan	18	-	**18**
Feb	9	-	**9**
Mar	1	120	**121**
Apr	17	92	**109**
May	65	50	**115**
Jun	55	40	**95**
Jul	47	56	**103**
Aug	56	17	**73**
Sep	122	35	**157**
Oct	164	24	**188**
Nov	81	16	**97**
Dec	42	23	**65**
TOTAL	**677**	**473**	**1,150**

Table 3
Infection of the swimbladder parasite *Anguillicoloides crassus* in yellow and silver life history stages of American eel (*Anguilla rostrata*) from South Carolina. Eels were screened for L_3 and L_4 larval parasites in the swimbladder wall ('Larval'), and adult parasites ('Adult') in the swimbladder lumen ('Total' refers to infection by any of the parasite stages). Prevalence: percent of eel infected; Abundance: mean number of parasites per eel; Intensity: mean number of parasites per infected eel.

	Eels screened			Prevalence			Abundance			Intensity		
System	Larval	Adult	Total	Larval	Adult	Total	Larval	Adult	Total	Larval	Adult	Total
Ashley	98	103	98	50%	65%	79%	2.7	3.1	5.8	5.4	4.7	7.4
Combahee	98	102	98	29%	48%	56%	1.3	2.3	3.6	4.6	4.8	6.4
Congaree	-	1	0	-	0%	-	-	0.0	-	-	-	-
Cooper	143	151	142	48%	66%	77%	1.2	2.8	4.1	2.6	4.2	5.2
Edisto	36	44	36	22%	45%	44%	0.6	2.1	1.7	2.5	4.6	3.9
Great Pee Dee	11	19	11	36%	58%	55%	0.5	1.3	1.3	1.3	2.2	2.3
Little Pee Dee	134	134	134	13%	45%	51%	0.2	0.7	1.0	1.8	1.7	1.9
Sampit	7	7	7	29%	71%	71%	0.7	2.1	2.9	2.5	3.0	4.0
Santee	20	31	20	5%	55%	55%	0.1	1.0	0.9	1.0	1.9	1.5
Savannah	2	5	2	0%	40%	0%	0.0	2.2	0.0	-	5.5	-
Winyah	66	73	66	53%	55%	74%	1.7	2.0	3.7	3.2	3.7	5.0
TOTAL	**615**	**670**	**614**	**35%**	**55%**	**65%**	**1.2**	**2.1**	**3.2**	**3.5**	**3.8**	**5.0**

Table 4
Infection of the invasive swimbladder parasite *Anguillicoloides crassus* in juvenile (glass eel and elver) life history stages of American eel (*Anguilla rostrata*) from Goose Creek Reservoir, South Carolina. Eels were screened for L_3 and L_4 larval stages of the parasite in the swimbladder wall, and adult stages of the parasite in the swimbladder lumen. Prevalence (P) is the percent of eel infected, abundance (A) is the mean number of parasites per eel, and intensity (I) is the mean number of parasites per infected eel. [Table taken from Hein et al. 2015].

				A. crassus L3/L4 larvae			*A. crassus* adults			*A. crassus*, any stage		
Eel stage	**Pigment stage**	***n***	**Mean TL (mm)**	**P (%)**	**A**	**I**	**P (%)**	**A**	**I**	**P (%)**	**A**	**I**
Glass eel	1	23	54.0	0.0	0.00	–	0.0	0.00	–	0.0	0.00	–
	2	36	53.1	0.0	0.00	–	0.0	0.00	–	0.0	0.00	–
	3	37	53.9	0.0	0.00	–	0.0	0.00	–	0.0	0.00	–
	4	40	53.0	0.0	0.00	–	5.0	0.05	1.00	5.0	0.05	1.00
	5	51	56.8	0.0	0.00	–	7.8	0.12	1.50	7.8	0.12	1.50
	6	67	60.1	1.5	0.01	1.00	14.9	0.25	1.70	14.9	0.27	1.80
	7	20	58.3	0.0	0.00	–	30.0	0.30	1.00	30.0	0.30	1.00
Glass eel	All (1–7)	274	56.0	0.4	0.00	1.00	8.0	0.11	1.41	8.0	0.12	1.46
Elver	Complete	199	94.1	17.1	0.51	2.97	56.3	1.41	2.51	58.8	1.92	3.27
TOTAL	All	473	72.1	7.4	0.22	2.91	28.3	0.66	2.33	29.4	0.88	2.98

Glass eel pigment stages follow Haro and Krueger (1988).

Table 5
Fish species screened for the invasive eel parasite *Anguillicoloides crassus*. Fish were collected from 2 stretches of the Ashley River (A1, A2) and one stretch of the Cooper River (C). Four species were identified as paratenic hosts of the *A. crassus* parasite (silver perch, Highfin goby, spot and mummichog). [Data from Li et al. 2015].

			Number			SL (mm)			Prevalence (%)			Mean abundance		
Order	Species	Common name	A1	A2	C	A1	A2	C	A1	A2	C	A1	A2	C
Perciformes	***Bairdiella chrysoura***	**Silver perch**		7	5		8.4	8.4		**86%**	**80%**		**13.4**	**24.2**
	Eucinostomus gula	Jenny morjarra		14	3		4.9	6.2		0%	0%		0	0
	Gobionellus oceanicus	**Higfin goby**			13			8.8			**39%**			**35.9**
	Lagodon rhomboides	Pinfish		6	25		8.1	8.4		0%	0%		0	0
	Leiostomus xanthurus	**Spot**	8	8		8.5	7.7		**100%**	**88%**		**78.1**	**85.5**	
	Lepomis auritus	Redbreast sunfish	6			10.7			0%			0		
	Lepomis macrochirus	Bluegill	16	1		8.5	9.2		0%	0%		0	0	
	Lepomis microlophus	Redear sunfish	5			6.8			0%			0		
Cyprinodontiformes	***Fundulus heteroclitus***	**Mummichog**	2	2		6.1	6.5		**100%**	**100%**		**1.0**	**4.0**	
Atheriniformes	*Menidia menidia*	Atlantic silverside	2	2	5	5.2	4	4.5	0%	0%	0%	0	0	0
Cypriniformes	*Notemigonus crysoleuca*	Golden shiner		1			7.2			0%			0	
Pleuronectiformes	*Citharichthys spilopteru*	Bay whiff		4			8.4			0%			0	
	Trinectes maculatus	Hogchoker		1			6.2			0%			0	
Clupeiformes	*Anchoa mitchilli*	Bay anchovy		6	30		4.1	4.1		0%	0%		0	0
	Brevoortia tyrannus	Atlantic menhaden			1			5.1			0%			0
	Dorosoma petenense	Threadfin shad		1			5.5			0%			0	
Siluriformes	*Ictalurus furcatus*	Blue catfish		1			10.2			0%			0	
Mugiliformes	*Mugil cephalus*	Striped mullet		6			9.8			0%			0	

Table 6
Number of otoliths used for marginal increment analysis.

	Number of Increments							
Month	1	2	3	4	5	6	7+	**TOTAL**
Jan/Feb			2	2	2	3	3	**12**
Mar		2	5	2	1	1		**11**
Apr	1	2	8	3	7	2		**23**
May	1	5	8	3	2	2	2	**23**
Jun	2	7	12	4	6	1	2	**34**
Jul	6	6	4	8	7	4		**35**
Aug	2	1	3	11	7	9	5	**38**
Sep		10	17	19	3	13	12	**74**
Oct	2	8	18	12	12	5	8	**65**
Nov		3	4	2	1	2		**12**
Dec	2	3	3	6	5	1	1	**21**
TOTAL	**16**	**47**	**84**	**72**	**53**	**43**	**33**	**348**

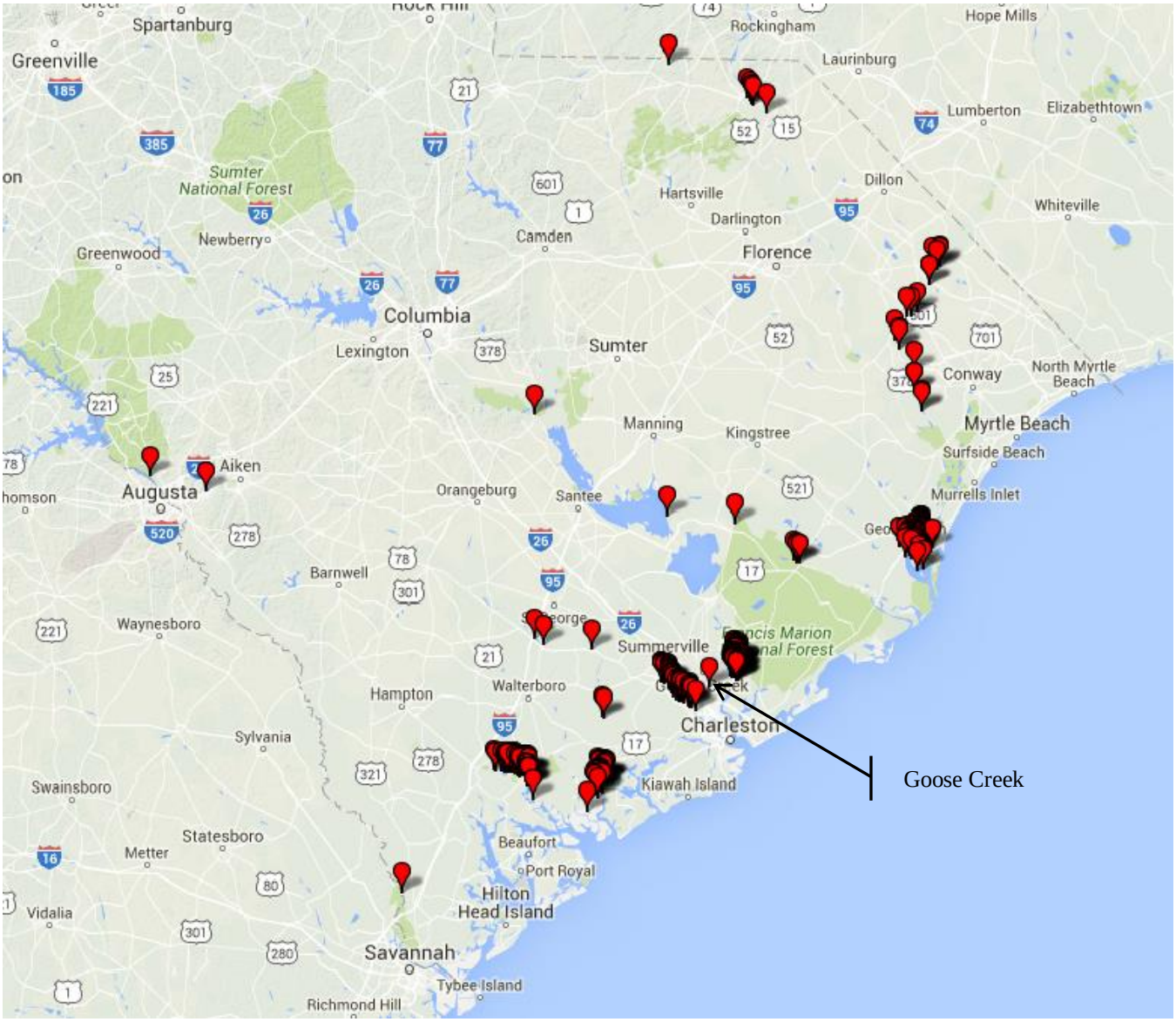

Figure 1
Map of all sites from where eels were collected. Glass eel and elver stage eels were collected from Goose Creek near North Charleston. Yellow and silver eel stages were collected from 183 sites spanning 11 river drainages.

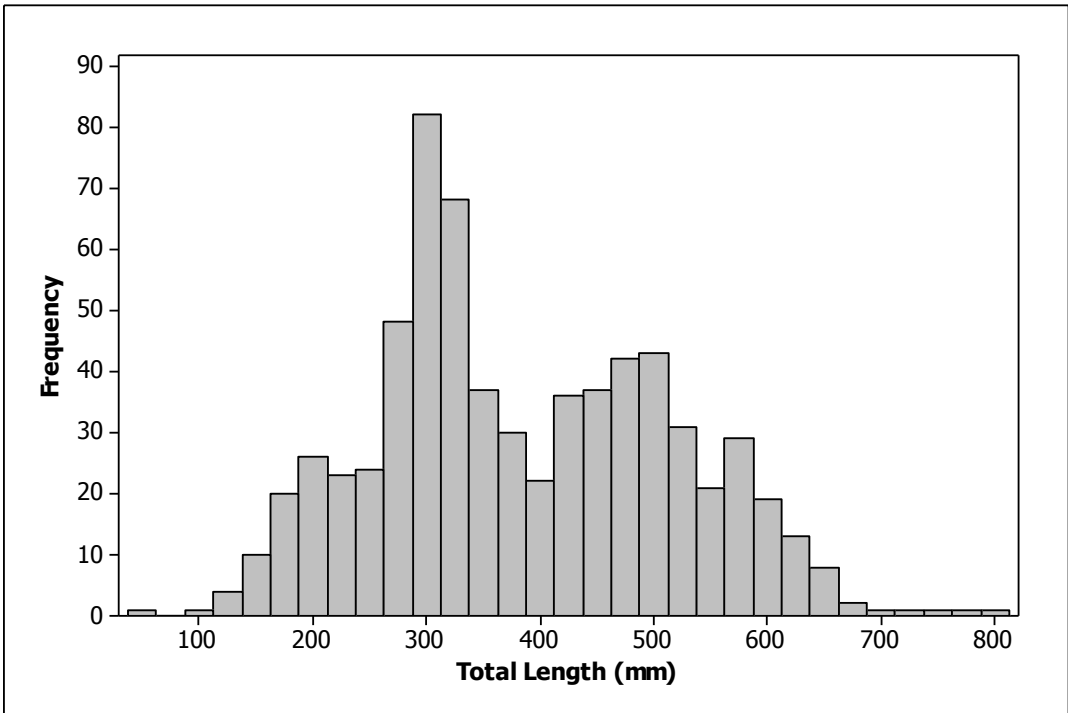

Figure 2
Total lengths of yellow and silver eels that were sampled from 11 rivers systems in South Carolina (n = 677).

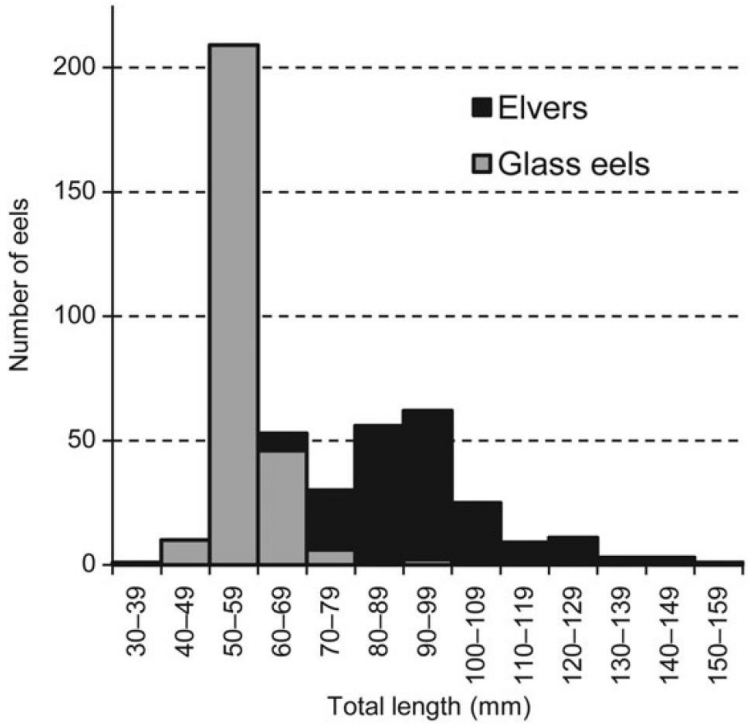

Figure 3
Total lengths of juvenile eels (glass eels and elvers) from Goose Creek reservoir, South Carolina (n = 473). [Figure from Hein et al. 2015].

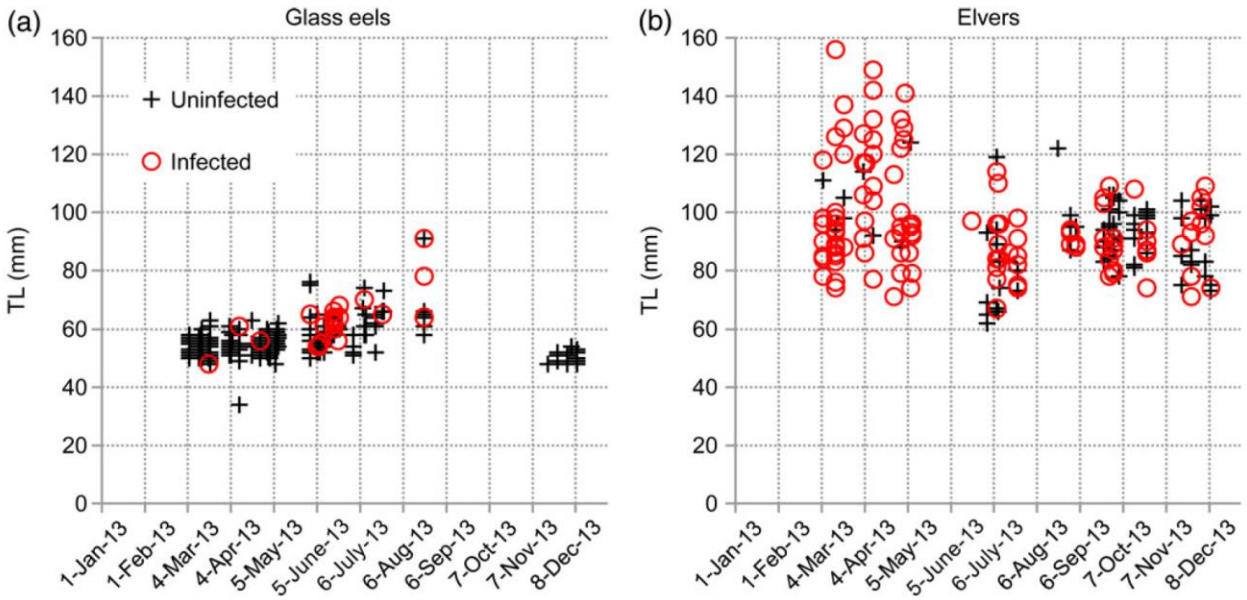

Figure 4
Infection by *A. crassus* parasites in *A. rostrata* glass eels (a) and elvers (b) collected from the South Carolina Goose Creek Reservoir eel ladder from March–December 2013. Black plus, uninfected; red circle, infected by *A. crassus* larval and/or adult stages. [Figure from Hein et al. 2015].

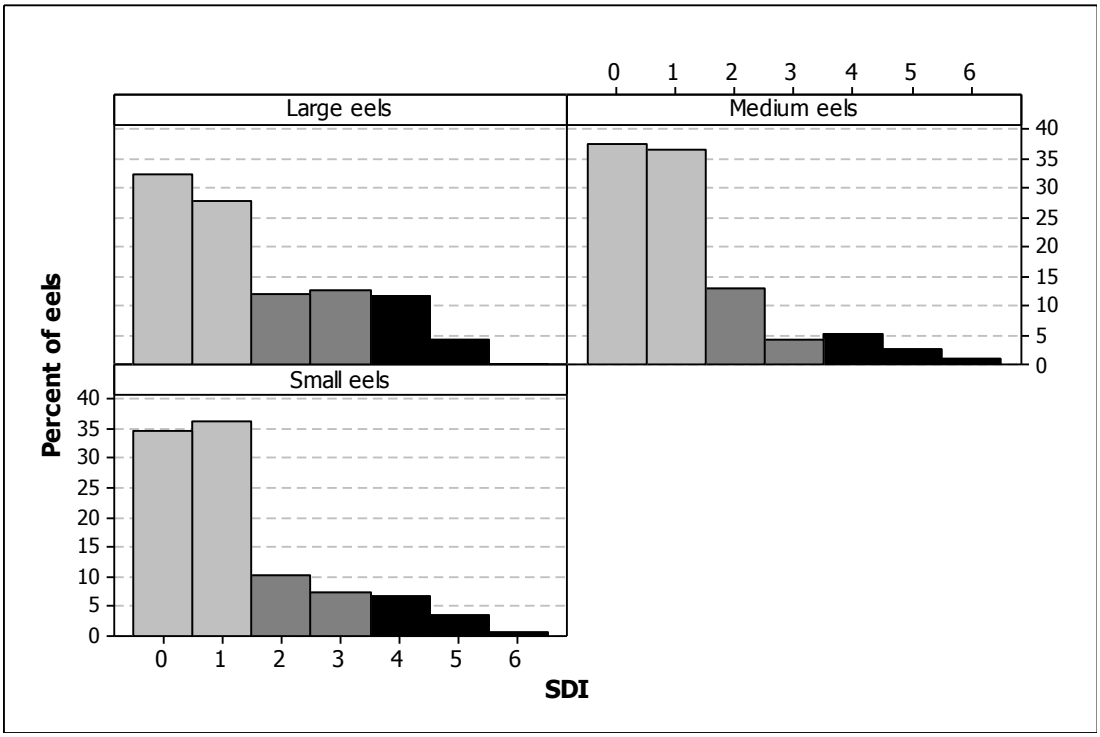

Figure 5

Frequency of swimbladder degenerative indices (SDI) in large, medium and small American eels from South Carolina. Damage to the swimbladder was either light (SDI = 0-1; light gray bars), moderate (SDI = 2-3; dark gray bars), or severe (SDI ≥ 4; black bars).

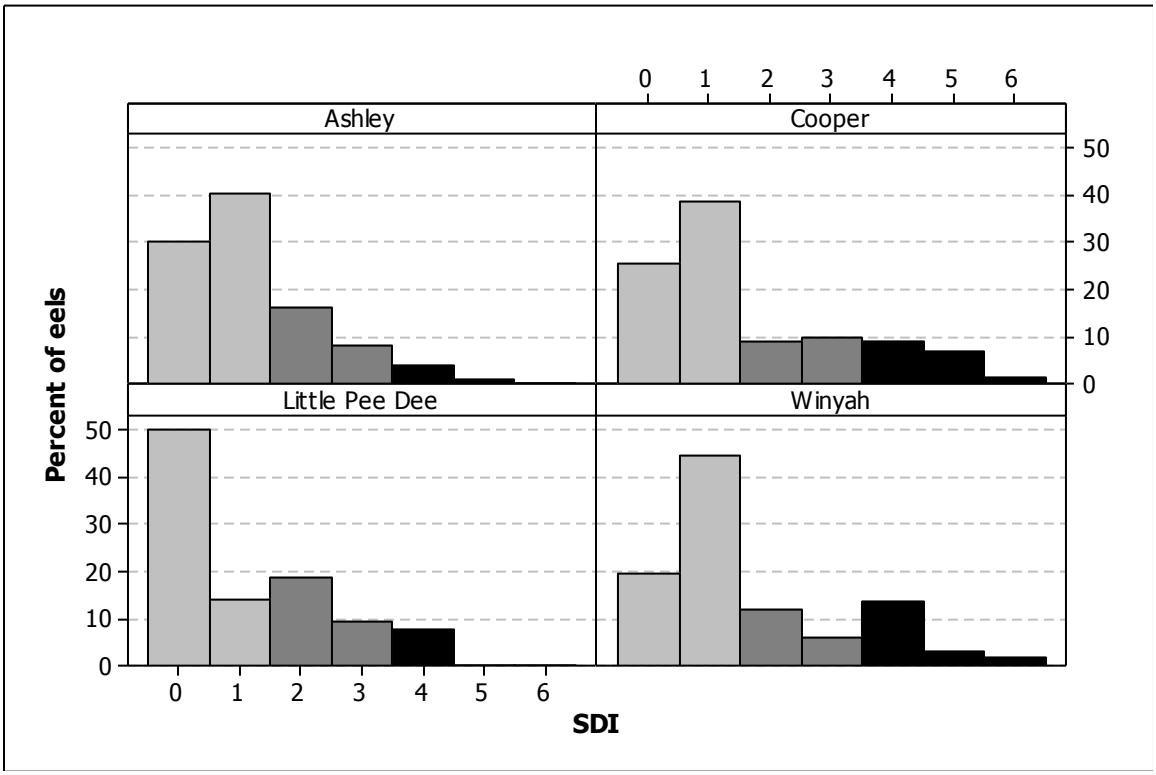

Figure 6
Frequency of swimbladder degenerative indices (SDI) in American eels from four of the river systems studied in South Carolina. Damage to the swimbladder was either light (SDI = 0-1; light gray bars), moderate (SDI = 2-3; dark gray bars), or severe (SDI ≥ 4; black bars).

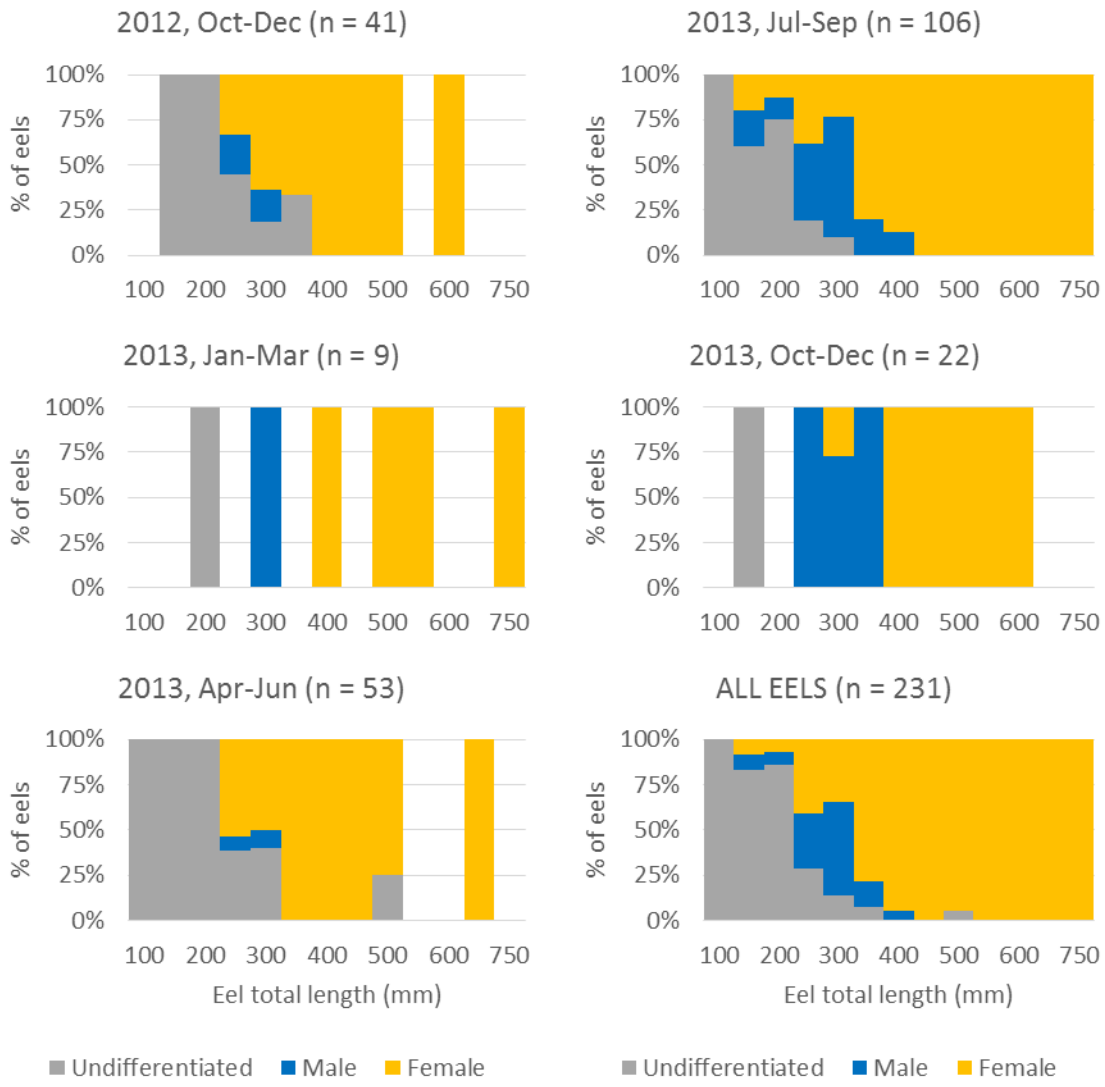

Figure 7
Sex ratio of American eels in South Carolina. Plots show the proportion of eels, in 50 mm length categories, that were either undifferentiated (gray), male (blue) or female (yellow). Males were generally smaller than females and less frequent than females, especially during April-June.

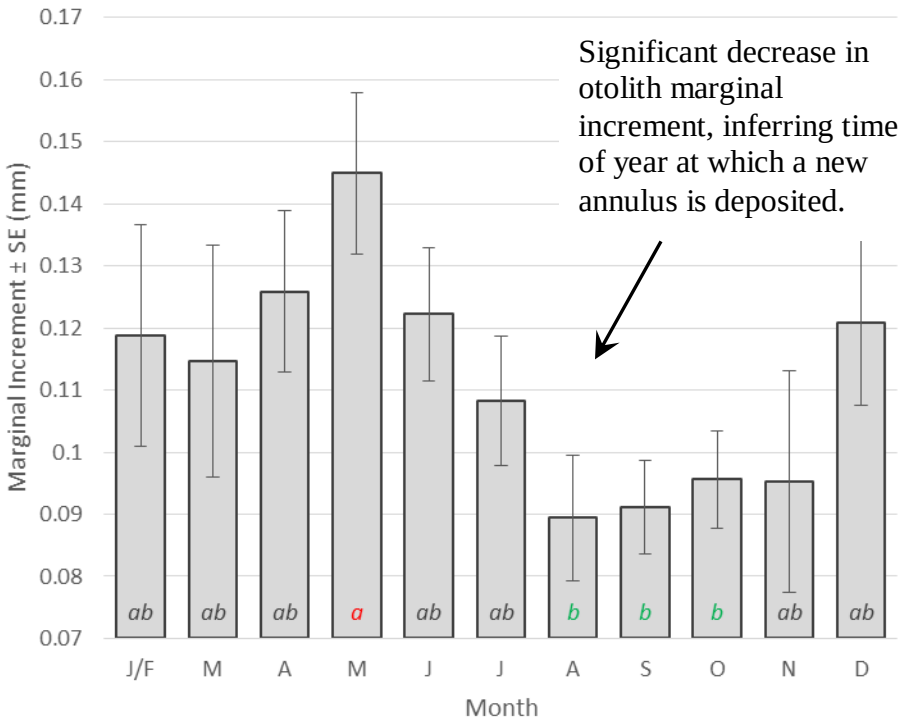

Figure 8
Marginal increment analysis of American eel otoliths confirming that just a single decrease in marginal increment width occurs during the year. Marginal increment values are least squares means ± SE from a general linear model controlling for otolith annuli count. Bars that do not share the same letter (italics) are significantly different from one another (Tukey post hoc pairwise comparisons, $p < 0.05$).

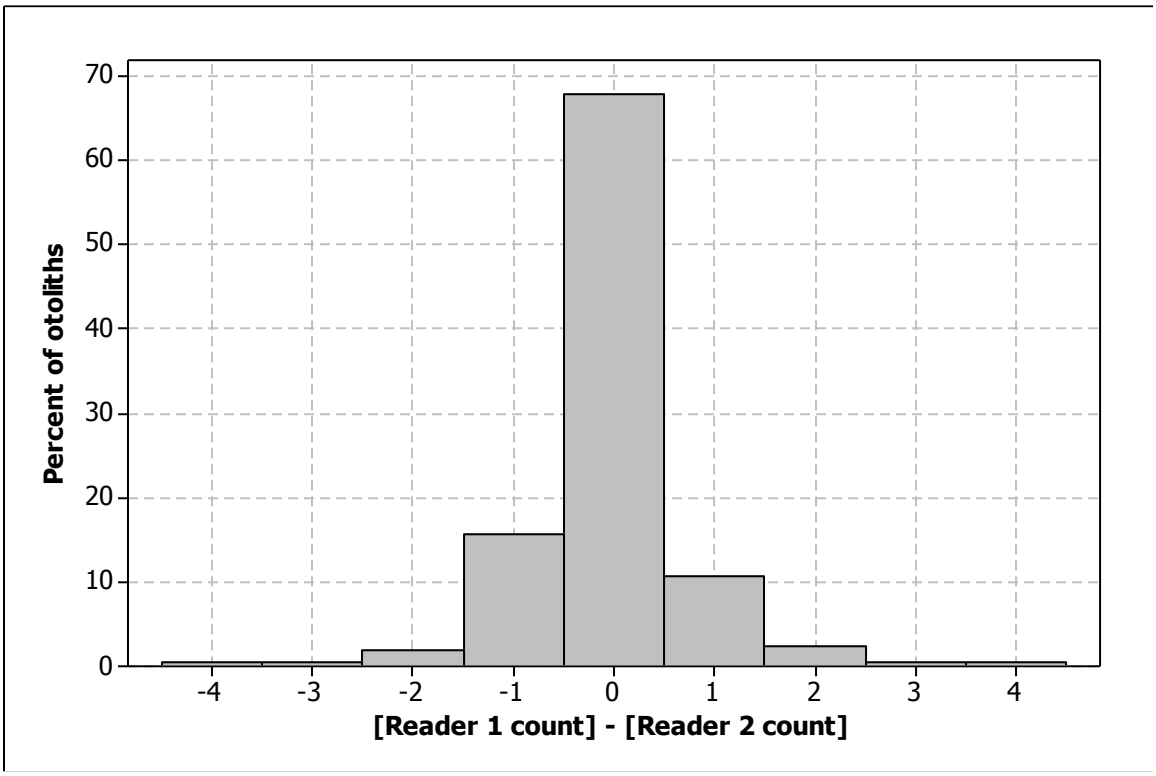

Figure 9
Difference in agreement of annuli counts by two independent otolith readers.

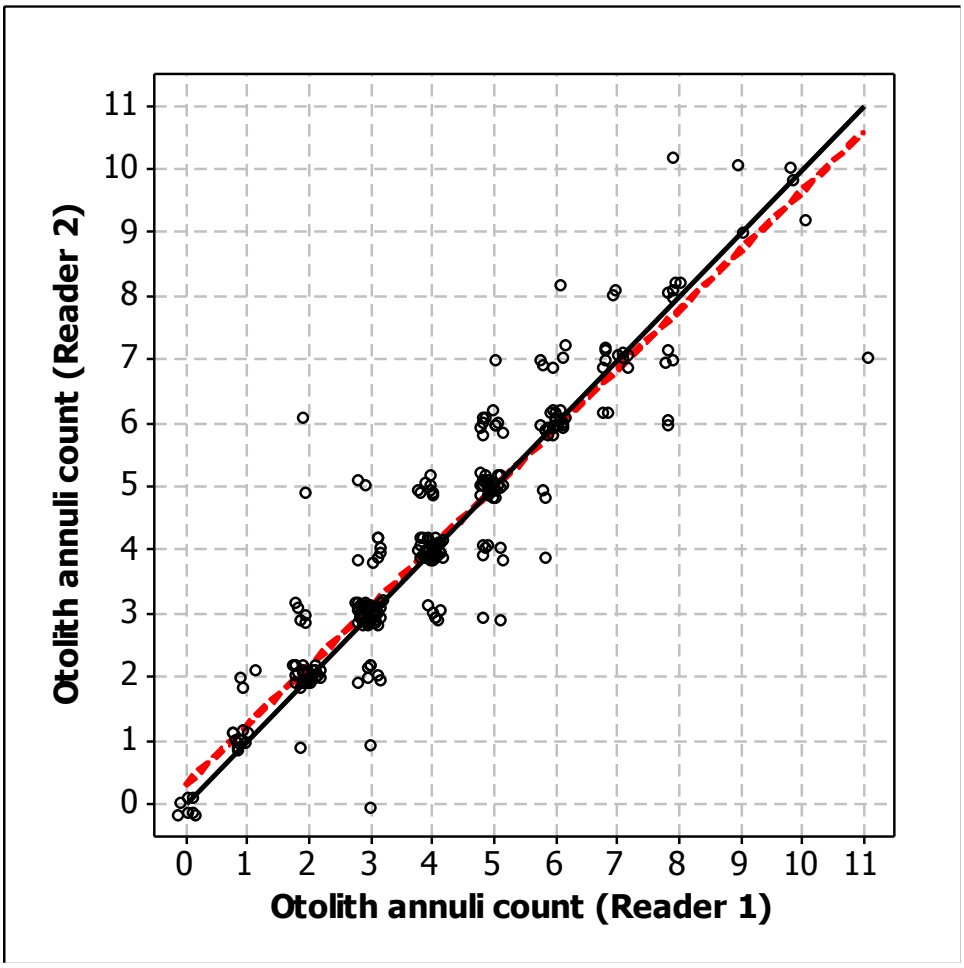

Figure 10
Comparison of annuli counts by two independent otolith readers (values jittered to offset overlapping data). Red line: linear regression fitted to data; Black line: 1:1 relationship.

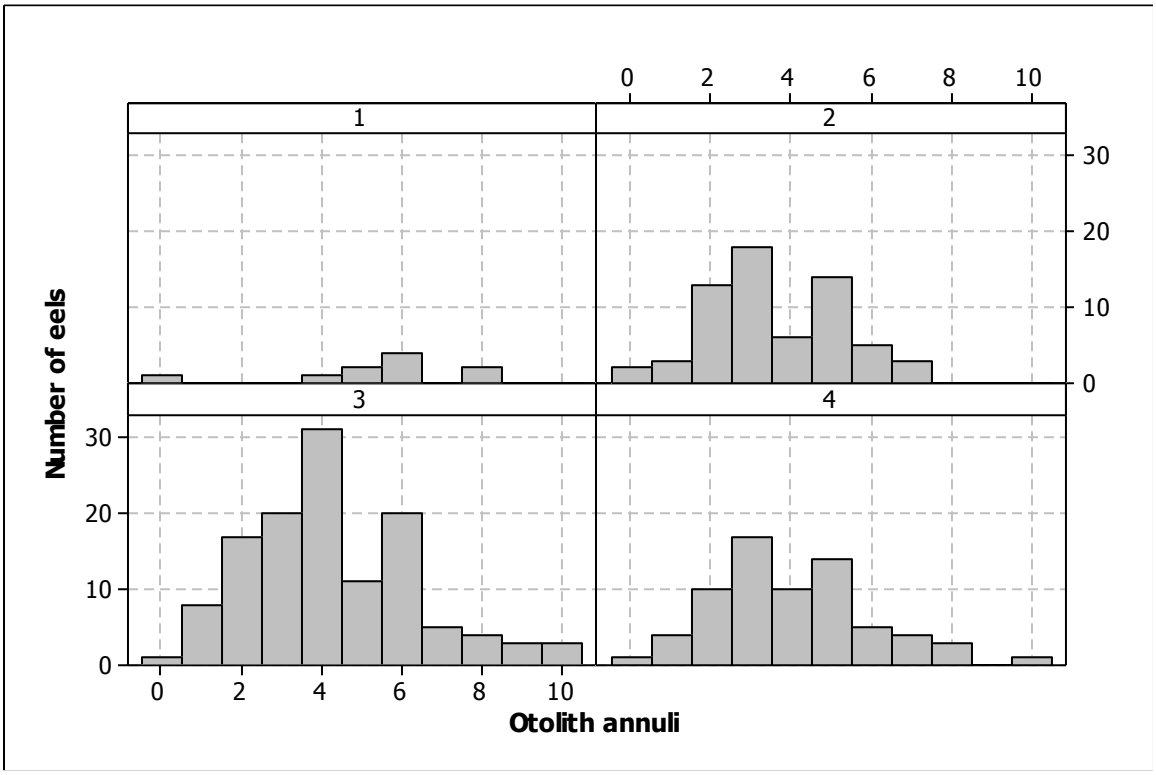

Figure 11
Distribution of annuli counts (age proxy) in different quarters of the year in American eels from South Carolina.

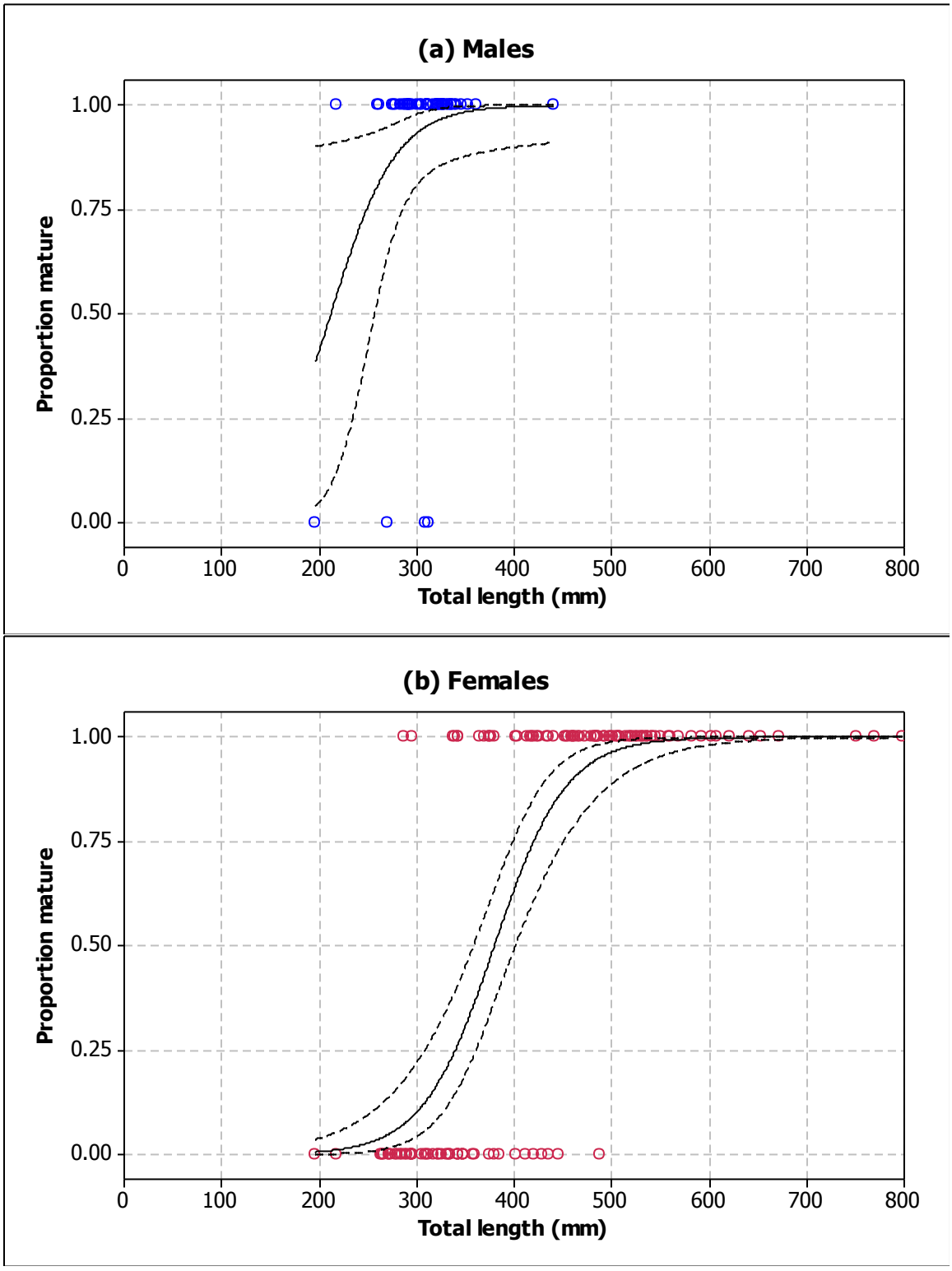

Figure 12
Relationship between length and maturity in (a) male and (b) female American eels from South Carolina. Dots represent individual eels (0: immature; 1: mature). Fitted lines (unbroken) are the fitted logistic model with 95% confidence limits (dash lines) across the range of sampled eel sizes.

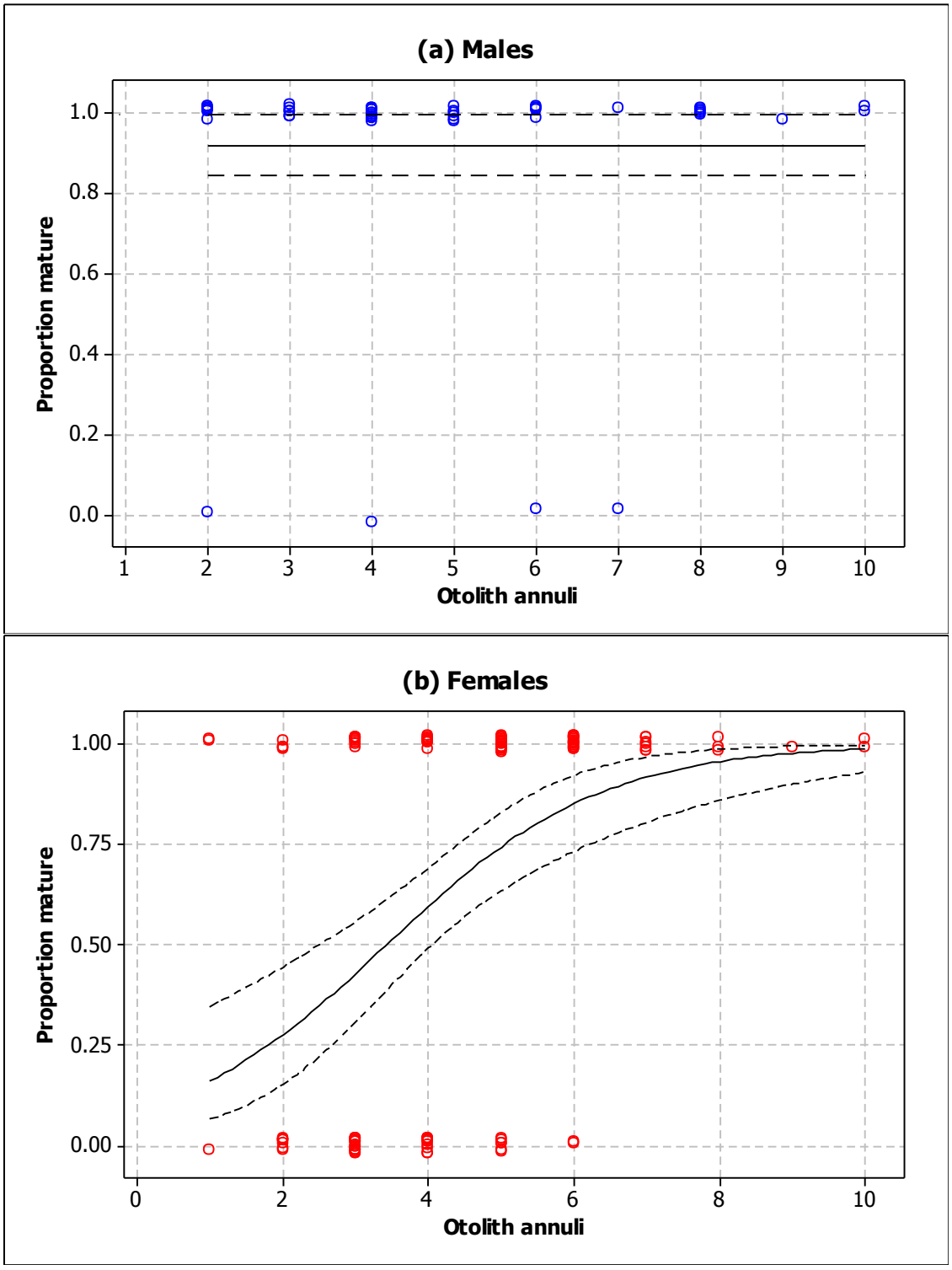

Figure 13
Relationship between otolith annuli (a proxy for age) and maturity in (a) male and (b) female American eels from South Carolina. Dots represent individual eels (0: immature; 1: mature; date jittered vertically to reduce overlap). For males, there was no significant relationship with annuli. For females, the fitted line (unbroken) is the fitted logistic model. (Dash lines: 95% confidence limits).

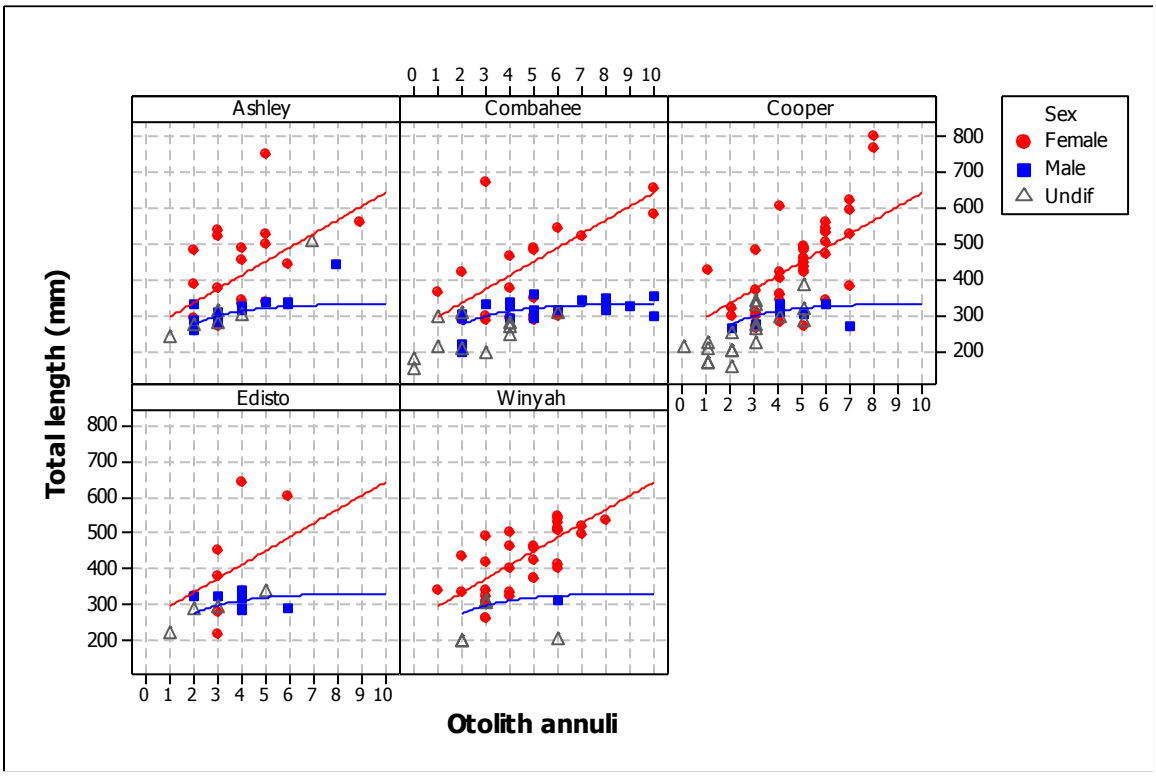

Figure 14
Relationships between total length and otolith annuli (age proxy) in male, female and undifferentiated American eels from five river systems in South Carolina. The two von Bertalanffy growth curves (red: females; blue: males) were fit to data pooled across rivers systems.